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Effects of irradiance-sulphur interactions on enzymes of carbon, nitrogen, and sulphur metabolism in maize plants

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Abstract

The effect of sulphur deprivation and irradiance (180 and 750 μmol m^{-2} s^{-1}) on plant growth and enzyme activities of carbon, nitrogen, and sulphur metabolism were studied in maize (*Zea mays* L. Pioneer cv. Latina) plants over a 15-d-period of growth. Increase in irradiance resulted in an enhancement of several enzyme activities and generally accelerated the development of S deficiency. ATP sulphurylase (ATPs; EC 2.7.7.4) and *o*-acetylserine sulphydrylase (OASs; EC 4.2.99.8) showed a particular and different pattern as both enzymes exhibited maximum activity after 10 d from the beginning of deprivation period. Hence in maize leaves the enzymes of C, N, and S metabolism were differently regulated during the leaf development by irradiance and sulphur starvation.

Additional key words: *o*-acetylserine sulphydrylase; ATP sulphurylase; chlorophyll; dry mass; glutamine synthetase; NAD-dependent glutamate dehydrogenase; nitrate reductase; phosphoenolpyruvate carboxylase; proteins; ribulose-1,5-bisphosphate carboxylase.

Introduction

The increasing interest in the effect of environmental factors on plant growth and development has led to investigation of irradiance-sulphur interactions and their effects on plant metabolism for a better understanding of plant responses to changes of irradiance and nutrient supply. Plants harvest radiant energy by oxygenic photosynthesis, so irradiance is a major factor influencing plant growth and productivity. Radiant energy supplies ATP and NADPH needed for CO_2 assimilation and regulates the activity of certain enzymes of reductive pentose phosphate cycle, the two additional enzymes of C_4-dicarboxylic acid CO_2 fixation pathway of C_4 plants, and enzymes of glucose oxidation. Many metabolic processes in higher plant tissues are stimulated by light as a result of a requirement for products of thylakoid electron transport or products of CO_2 assimilation. Sulphur is a macronutrient of plants that is required for protein synthesis. Besides, S is a structural constituent of several coenzymes and prosthetic groups, such as ferredoxin, which are important for nitrogen assimilation (Petrovic and Kastori 1994, Marschner 1995). Thus, S plays an important role in plant growth and in the regulation of plant development (Schwenn and Trebst 1976). It is available to plants mainly in the form of sulphate (Rennenberg 1984) from the soil. Sulphur is a relatively immobile element in plants (Salisbury and Ross 1992). However, endogenous S is redistributed from mature leaves of several S-sufficient plants (Herschbach and Rennenberg 1994). Glutathione is the most abundant long-distant transport form of reduced sulphur (Herschbach and Rennenberg 1997).

The effect of S nutrition on S redistribution and the effects of S starvation have received large attention, but little research has so far focused on the interactions between irradiance and S deficiency. In order to examine the effect of both factors, maize (*Zea mays*) plants were grown under two different irradiances and were transferred to S-deprived nutrient solution 12 d after sowing. We investigated the activity of some enzymes involved in carbon, nitrogen, and sulphur metabolism at different time from the beginning of S-starvation period.

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Abbreviations: ATPs, ATP sulphurylase; BSA, bovine serum albumin; Chl, chlorophyll; DM, dry mass; DTT, DL-dithiothreitol; EDTA, ethylenediaminetetracetic acid; GDH, NAD-dependent glutamate dehydrogenase; GS, glutamine synthetase; HEPES, 4-(2-hydroxyethyl)-1-piperazine ethanesulphonic acid; HI, high irradiance; LI, low irradiance; NR, nitrate reductase; OASs, *o*-acetylserine sulphydrylase; PEPC, phosphoenolpyruvate carboxylase; PMSF, phenylmethylsulphonyl fluoride; PVP, polyvinylpyrrolidone; RuBPC, ribulose-1,5-bisphosphate carboxylase.

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Materials and methods

Plants: Maize seeds (*Zea mays* L. Pioneer cv. Latina) were imbibed for 24 h in distilled water and then allowed for 3 d to germinate in moistened paper rolls in the dark at 28 °C. Seedlings with roots were transferred to plastic jars (35 plants per jar) containing 1 200 cm^3 of nutrient solution of Hoagland and Arnon (1950). The nutrient solution was continuously aerated and renewed every 3 d. Seedlings were grown in a climate chamber (day/night: relative humidity 70/80 %, temperature 27/20 °C, 14/10 h light/dark photoperiod) under 180 (LI) and 750 (HI) μmol m^{-2} s^{-1} PAR at leaf level. After 12 d, half of the plants were transferred in a *minus* sulphate solution. Sulphate salts (Mg^{2+}, NH_4^+, Fe^{2+}, Mn^{2+}, Zn^{2+}, Cu^{2+}) were replaced by appropriate amounts of chloride salts (Mg^{2+}, NH_4^+, Fe^{2+}, Mn^{2+}, Zn^{2+}, Cu^{2+}). After 0, 3, 7, 10, and 15 d from the beginning of S-deprivation period, the third leaf of each plant was excised, frozen in N_2, and stored at -80 °C until used.

Enzyme extraction and assays: Frozen tissue (*ca.* 1 g of fresh mass) was ground to a fine powder in a pre-chilled mortar under liquid N_2. Cold extraction buffer containing 50 mM HEPES-KOH (pH 7.4), 5 mM $MgCl_2$, 1 mM EDTA, 10 % (v/v) glycerol, 0.1 % (v/v) *Triton X-100*, 5 mM DTT, 1 mM PMSF, and 1 % (m/v) PVP was added in a ratio of 1 : 7 (m/v). The brei was filtered through four layers of cheesecloth and the homogenate was centrifuged at 1 000×*g* for 5 min at 4 °C. The resulting supernatant was desalted at 4 °C on a *Sephadex G-25* column (*PD-10*, *Pharmacia*, Uppsala, Sweden) pre-equilibrated with extraction buffer minus *Triton X-100*. The desalted extract was then centrifuged at 30 000×*g* for 5 min at 4 °C. The supernatant was divided into 300 mm^3 aliquots which were frozen in liquid N_2 and stored at –80 °C until analysis.

Phosphoenolpyruvate carboxylase (PEPC; EC 4.1.1.31) activity was assayed spectrophotometrically at 340 nm in a final volume of 1 cm^3 containing 50 mM HEPES-KOH (pH 8.0), 10 mM $MgCl_2$, 5 mM $NaHCO_3$, 0.2 mM NADH, 5 units of malate dehydrogenase (EC 1.1.1.37), starting the reaction with 2.5 mM PEP. Ribulose-1,5-bisphosphate carboxylase (RuBPC; EC 4.1.1.39) activity was determined by incubation of leaf extract with 10 mM $MgCl_2$ and 20 mM $NaHCO_3$ for 5 min, and total activity was measured spectrophotometrically following the formation of 3-phosphoglycerate (Lea 1990). Nitrate reductase (NR; EC 1.6.6.1) activity was determined according to Neyra and Hageman (1975) with 5 mM Mg^{2+} in the assay mixture. Glutamine synthetase (GS; EC 6.3.1.2) activity was determined as described in Lea (1990). The activity of NAD-dependent glutamate dehydrogenase (GDH; EC 1.4.1.2) was assayed spectrophotometrically at 340 nm in a final volume of 1 cm^3 containing 70 mM Tris-HCl (pH 8.1), 1 mM $CaCl_2$, 0.1 mM NADH, 150 mM NH_4-acetate, and by starting the reaction with 20 mM 2-oxoglutarate. For ATP sulphurylase (ATPs; EC 2.7.7.4) and *o*-acetylserine sulphydrylase (OASs; EC 4.2.99.8) the procedures described by Ferretti *et al.* (1993) were followed. All reported activities were measured in duplicate and were linear with time and proportional to the amount of extract used.

Other measurements and statistics: Protein content was determined according to Bradford (1976) using BSA as standard. Chlorophyll (Chl) contents were determined according to Wintermans and De Mots (1965). To determine sulphur content, 1 g of leaf sample, dried at 105 °C, was ashed in a muffle furnace at 600 °C. The ash was dissolved in 10 cm^3 of 3 M HCl and filtered through *Whatman* No. *42* paper. In contact with $BaCl_2$, a $BaSO_4$ precipitate is formed which is determined turbidimetrically (Bardsley and Lancaster 1962).

Values are means from an experiment run in triplicate representative of four independent experiments. S.D. did not exceed 5 % of the means.

Results

Maize plants grown under the two irradiances reached the same developmental stage at harvest. However, HI plants produced higher amounts of dry matter (+150 %) than the ones under LI (Fig. 1*A*). Dry mass increased as leaf aged (+104 and +177 % in HI and LI leaves, respectively). Sulphur deficiency allowed plant growth, but the increase in dry matter was lower.

After 12 d from sowing, HI leaves showed a higher (+37 %) sulphur content (Fig. 1*B*) than the LI ones. Sulphur content increased during the following 15 d and the difference between HI and LI plants did not change. As expected, concentrations of S in leaves were much lower in S-deficient than in S-sufficient plants, but were dependent on irradiance. After 15 d of S-deprivation period this parameter was 55 and 78 % lower than in HI and LI control plants, respectively.

HI severely decreased Chl content (Fig. 1*C*). After 12 d from sowing, Chl content of HI leaves was 42 % lower than that of LI leaves. The difference in Chl content between the two irradiances subsequently increased either with leaf age or S deprivation. After the following 15 d of growth the difference was 52 % between HI and LI control leaves and 77 % between HI and LI S-starved leaves.

Protein content was slightly influenced by the irradiance of growth (Fig. 1*D*), but strongly decreased in S-deprived plants (42 and 57 % lower than control under HI and LI, respectively) after 15 d of sulphur deficiency.

PEPC activity (Fig. 2*A*) was increased by HI, but was significantly reduced by leaf ageing and sulphur starvation. The reduction was higher in HI plants and was faster in S-deprived plants. RuBPC activity (Fig. 2*B*) was also affected by irradiance. Under HI, RuBPC activity decreased by leaf ageing and S-starvation, whereas under LI the activity increased as leaves aged and S-deprivation proceeded. The increment was higher in S-deprived plants.

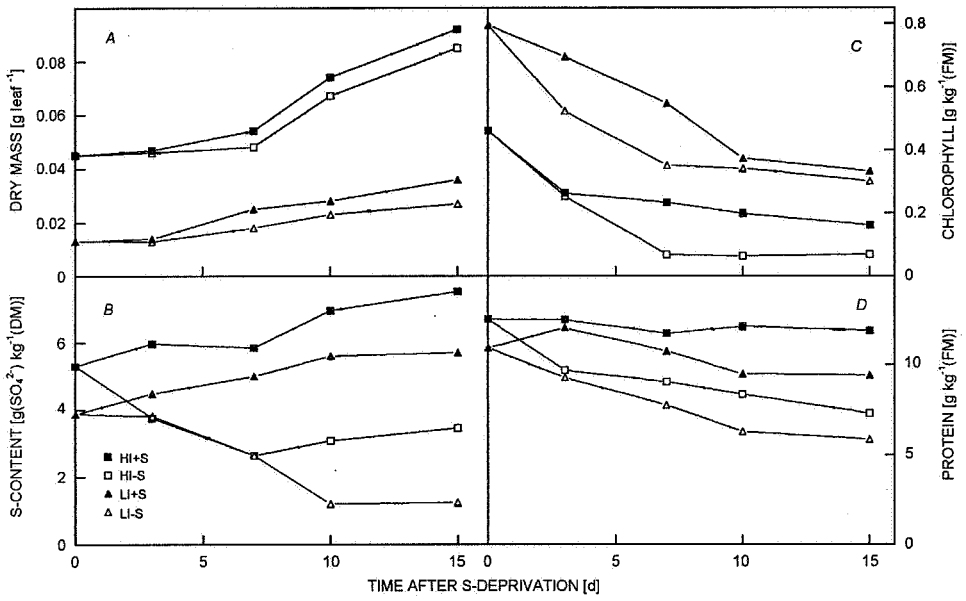

Fig. 1. Effects of S-deprivation on dry mass (*A*) and contents of total sulphur (*B*), chlorophyll (*C*), and protein (*D*) in third leaf of maize plants grown under different irradiance at different times of S-deprivation. Means from an experiment run in triplicate representative of four independent preparations. S.D. did not exceed 5 % of the means.

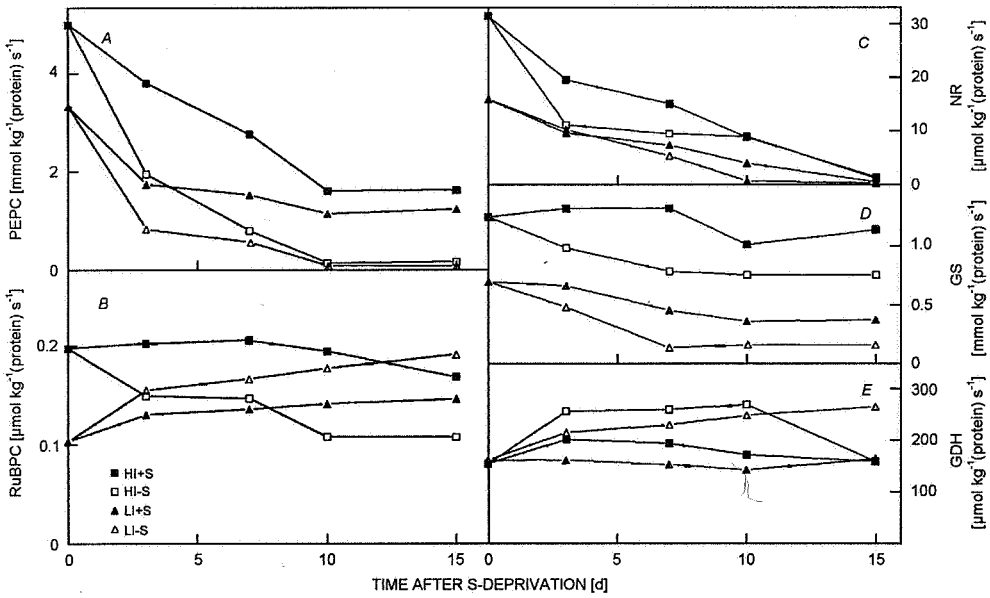

Fig. 2. Activities of PEPC (*A*), RuBPC (*B*), NR (*C*), GS (*D*), and GDH (*E*) in maize leaves exposed to S-starvation and different irradiance. Means from an experiment run in triplicate representative of four independent preparations. S.D. did not exceed 5 % of the means.

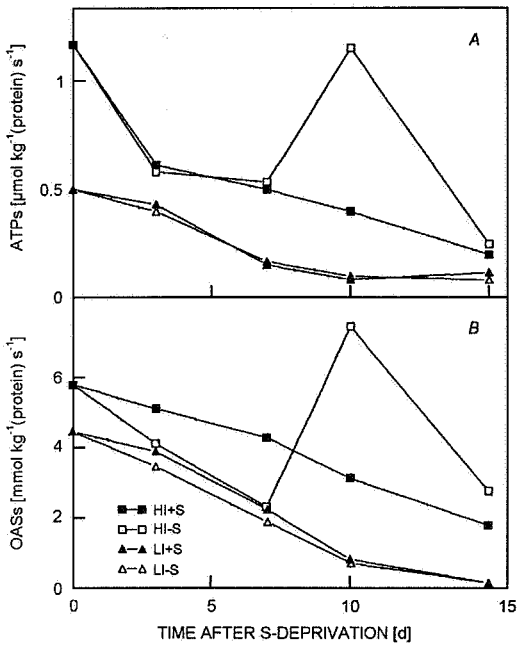

Fig. 3. Effect of removal of S supply on ATPs (*A*) and OASs (*B*) activities of third leaf of maize grown under two irradiances. Means from an experiment run in triplicate representative of four independent preparations. S.D. did not exceed 5 % of the means.

In leaves under HI, NR activity was 98 % higher than in ones under LI (Fig. 2*C*). NR activity rapidly decreased, reaching values near to zero in the following 15 d. The loss of activity was more pronounced under S-deficiency and the response was faster in HI leaves. GS, the major enzyme in ammonia assimilation, responded positively to irradiance, showing in HI leaves an activity 78 % higher than in LI leaves (Fig. 2*D*). As leaf aged, GS activity was slightly influenced in HI plants, whereas it decreased (-46 %) in LI plants. Therefore, the loss of activity was not so fast as NR activity. Furthermore, after 15 d of S-deprivation period, GS activity was 33 and 77 % lower than control under HI and LI, respectively. GDH activity responded to a lesser extent than NR and GS activities to irradiance and leaf ageing (Fig. 2*E*). Sulphur deficiency produced an increase of the same activity but the patterns were dependent on irradiance: HI leaves showed the maximum activity after 10 d of S-deprivation and afterwards declined, whereas LI leaves exhibited an increasing activity during 15 d of S-deprivation.

After 12 d from sowing, leaf extractable activity of ATPs was more affected (+140 %) by the irradiance of growth than OASs (+29 %) (Fig. 3*A,B*). During the following 15 d, both activities strongly decreased in control plants reaching values near to zero and the pattern was independent on irradiance. On the other hand, S-deficiency produced an increase of ATPs and OASs activities which reached a transient maximum value after 10 d of S-starvation (+188 and +143 %, respectively) under HI.

Discussion

Our results demonstrate that several metabolic processes are sensitive to changes of irradiance and S-deprivation. HI treatment resulted in an increase of dry mass and activity of the first enzymes of C, N, and S metabolic pathways. We found that HI and leaf ageing produced higher amounts of dry matter. A similar effect of irradiance and leaf ageing was shown in S content of maize leaves. Furthermore, decrease of S content induced by S deficiency was particularly remarkable in HI plants. Inhibition of plant growth by S starvation was not particularly notable when the plants were grown under both HI and LI suggesting that in our experimental conditions the shortage of sulphur was not inhibiting plant growth. Chl content in leaves showed a decrease by HI, leaf ageing, and S deficiency. The decrease in Chl content and in the Chl *a/b* ratio (values not shown) suggests an induction by HI of premature senescence and an increase in senescing rate by S deficiency.

HI plants showed an increase in all measured enzyme activities. NR and ATPs activity showed highest increase (+126 and +176 %, respectively). NR is the key enzyme of nitrate assimilation pathway and its control plays a critical role in the regulation of N assimilation (Oaks 1993, Campbell 1996, 1999). Furthermore, the light-modulation of NR activity in maize tissues has been observed (Remmler and Campbell 1986, Merlo *et al.* 1995). The activity of ATPs, the first enzyme of sulphate assimilation pathway, was more affected by the irradiance of growth than OASs, probably due to the role of this enzyme in the production of both reduced sulphur and sulpholipids (Marschner 1995). Our results indicate that irradiance affects enzymes of C, N, and S assimilation. However, key enzymes in CO_2, nitrate, and sulphate assimilation (RuBPC, NR, and ATPs, respectively) were differently influenced by irradiance. Leaf ageing induced a decrease of all measured parameters and the decrease was particularly rapid in the first week of the experimental period. S-starvation probably induces premature senescence within the leaves of maize plants. A further reduction in the amounts of dry mass, Chl, and proteins in leaves of S-deprived plants was found. The most remarkable decrease was in HI plants. Moreover, all enzyme activities, with minor exceptions, were reduced by S-starvation.

The increase of RuBPC activity from leaves of LI plants was an unexpected result. However, leaves can sometimes lose much of their RuBPCO-protein content at low irradiance, without affecting their photosynthetic

capacity (Quick *et al.* 1991, 1992). Enzymes involved in N assimilation pathway were differently affected by S-starvation treatment. NR and GS activities were reduced while GDH activity was enhanced by deprivation of sulphur. The different behaviour of GDH might be explained assuming that GDH plays no role in ammonium assimilation and is active in the catabolism of glutamate in higher plants in response to a deficiency of carbon (Robison *et al.* 1991). This finding, accomplished by decrease of Chl and protein contents, supports the theory of a too early ageing induced by S deprivation. Enzymes of S metabolism showed a particular behaviour in response to S deficiency. HI plants showed an increase both of ATPs and OASs activities (+184 and +143 %, respectively) after 10 d of S-deprivation. Thus our results clearly demonstrate that the maximum activity of enzymes involved in S metabolism can only be achieved at specific sulphur content in the leaves.

We found that exposure to HI results in an increase of all measured parameters as well as in a decrease of Chl content. The major effect of HI occurred in the activity of the key enzymes of the three considered metabolisms. A number of observed effects induced by S starvation treatment resemble the ageing phenomena, *i.e.*, the lower Chl and protein content, higher GDH activity, and activity of the enzymes involved in C, N, and S metabolism. Finally, S deprivation negatively affects enzyme activities. In response to S starvation, plants showed an increase of ATPs and OASs activities when critical concentration of sulphur was achieved.

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